

Synthesis and larvicidal and adult topical activity of some hydrazide-hydrazone derivatives against *Aedes aegypti*

Kadriye AKDAG, Bedia KOCYIGIT-KAYMAKCIOGLU, Nurhayat TABANCA, Abbas ALI, Alden ESTEP, James J. BECNEL, Ikhlas A. KHAN

ABSTRACT

A series of novel hydrazide-hydrazone derivatives were synthesized and evaluated for their larvicidal and adult topical activity against *Aedes aegypti*. The proposed structures of all the synthesized compounds were confirmed using elemental analysis, UV, IR, ¹H-NMR, ¹³C-NMR and mass spectroscopy. Compounds **4a-h** were screened in larval bioassays at concentrations of 100, 50 and 25 ppm in a dose dependent manner and data for their mortality was recorded. Among the tested compounds, 1-(4-nitrophenyl)-3-(4-{2-

oxo-2-[2-(2-oxo-1,2-dihydro-3H-ylidene)hydrazinyl] ethyl} phenyl)urea (**4b**) showed noteworthy larvicidal activity against *Aedes aegypti*. Dose-response data of compound **4b** showed LC₅₀ and LC₉₀ values of 30.5 (15.4 – 22.7) and 95.9 (73.8 – 139.4) ppm, respectively. Screening of compounds **4a-h** at four doses by topical bioassay indicated a range of activity between 10000 and 1000 ppm.

Keywords: Hydrazide-hydrazone, mosquito control, *Aedes aegypti*, larvicidal activity, adult topical activity

INTRODUCTION

Mosquitoes are vectors of serious human pathogens that cause malaria, Dengue Fever, Yellow Fever, Rift Valley Fever, and Chikungunya. All of these diseases can lead to explosive outbreaks in humans which can cause high rates of morbidity and mortality [1-3]. These mosquito vector-borne diseases are ecologically sensitive; continue to be endemic in regions with human interactions leading to introductions in new geographical regions and potential epidemics in the future [2]. Currently, the development of naturally occurring insecticides and repellents are under exploration to increase and improve our ability to protect humans from mosquito bites, and ultimately to reduce the incidence of mosquito-borne illnesses. Personal protection and control mosquitoes in larval stages are the general accomplishments for the mosquito control [4-5]. Larvicides play significant role in controlling mosquitoes at their breeding and immature stages. Hydrazone derivatives possessed good larvicidal activity in our previous study [6-8]. In recent years, the chemistry of carbon-nitrogen double bond of hydrazone is fast becoming the backbone of condensation reaction in benzo-fused N-heterocycles. Hydrazone containing azomethine –NHN=CH protons

Kadriye Akdag, Bedia Kocyigit-Kaymakcioglu
Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Marmara University, 34668, Istanbul, Turkey

Nurhayat Tabanca, Abbas Ali, Ikhlas A. Khan
Center for Natural Products Research, The University of Mississippi, 38677, Mississippi, USA

Alden Estep, James J. Becnel
USDA, ARS, Center for Medical, Agricultural and Veterinary Entomology (CMAVE), Gainesville, FL 32608, USA

Ikhlas A. Khan
Department of Pharmacognosy, School of Pharmacy, The University of Mississippi, 38677, Mississippi, USA

Alden Estep
Navy Entomology Center of Excellence, NASJAX, Jacksonville, Florida, USA

Corresponding author:

Bedia Koçyiğit-Kaymakcioğlu
Department of Pharmaceutical Chemistry, Faculty of Pharmacy,
Marmara University, Istanbul, 34668, Turkey
Tel: +90-212-4142963 Fax: +90-212-3452952
E-mail: bkaymakcioglu@marmara.edu.tr

Report Documentation Page			Form Approved OMB No. 0704-0188		
Public reporting burden for the collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to a penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number.					
1. REPORT DATE 2014	2. REPORT TYPE		3. DATES COVERED 00-00-2014 to 00-00-2014		
4. TITLE AND SUBTITLE Synthesis and larvicidal and adult topical activity of some hydrazide-hydrazone derivatives against Aedes aegypti			5a. CONTRACT NUMBER		
			5b. GRANT NUMBER		
			5c. PROGRAM ELEMENT NUMBER		
6. AUTHOR(S)			5d. PROJECT NUMBER		
			5e. TASK NUMBER		
			5f. WORK UNIT NUMBER		
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Navy Entomology Center of Excellence, PO Box 43, Naval Air Station, Jacksonville, FL, 32212			8. PERFORMING ORGANIZATION REPORT NUMBER		
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)			10. SPONSOR/MONITOR'S ACRONYM(S)		
			11. SPONSOR/MONITOR'S REPORT NUMBER(S)		
12. DISTRIBUTION/AVAILABILITY STATEMENT Approved for public release; distribution unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT A series of novel hydrazide-hydrazone derivatives were synthesized and evaluated for their larvicidal and adult topical activity against Aedes aegypti. The proposed structures of all the synthesized compounds were confirmed using elemental analysis, UV, IR, 1H-NMR, 13C-NMR and mass spectroscopy. Compounds 4a-h were screened in larval bioassays at concentrations of 100, 50 and 25 ppm in a dose dependent manner and data for their mortality was recorded. Among the tested compounds, 1-(4-nitrophenyl)-3-(4-{2-oxo-2-[2-(2-oxo-1,2-dihydro-3H-ylidene)hydrazinyl] ethyl} phenyl)urea (4b) showed noteworthy larvacidal activity against Aedes aegypti. Dose-response data of compound 4b showed LC50 and LC90 values of 30.5 (15.4 ? 22.7) and 95.9 (73.8 ? 139.4) ppm, respectively. Screening of compounds 4a-h at four doses by topical bioassay indicated a range of activity between 10000 and 1000 ppm.					
15. SUBJECT TERMS					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT Same as Report (SAR)	18. NUMBER OF PAGES 6	19a. NAME OF RESPONSIBLE PERSON
a. REPORT unclassified	b. ABSTRACT unclassified	c. THIS PAGE unclassified			

constitute an important class of compounds for new drug development [9-10].

Hydrazones are present in many of the bioactive heterocyclic compounds because of their various biological and clinical applications such as antimicrobial, antiplatelet, anticancer, antifungal, antiviral, antitumoral, antibacterial and antimalarial activities [11-13]. Recently, our group has been investigating the possible pharmacological potential of new molecules that contain a hydrazide-hydrazone scaffold [14-15].

Many hydrazone derivatives have been reported to possess broad spectrum insecticidal activity and are used as active ingredients for controlling agricultural and horticultural pests [16-18]. For example, the first hydrazone type insecticide, hydramethylnon, was registered for use in the United States by the Environmental Protection Agency in 1980 to control ants and cockroaches [18]. Aggarwal et al. [19] found that nalidixic hyrazide derivatives demonstrated 70-100% mortality against *Spodoptera litura*. Yang et al. [20] reported that cholesterol-based hydrazone derivatives showed better insecticide activity than their precursor cholesterol against pre-third larvae of *Mythimma separata* in vivo.

As part of our current project to discovering new insecticides for control of these vectors and to explore the bioactivity of hydrazone derivatives, 4-[[substitutedphenyl] carbamoyl]amino}phenyl)acetic acid derivatives were synthesized and tested for their larvicidal activity against 1-day old and adulticidal activity against 3-5 day old adult female *Aedes aegypti* L.

MATERIALS AND METHODS

Reactions were monitored by thin layer chromatography (TLC) and purity of the products was checked by High Performance Liquid Chromatography (HPLC). TLC was performed on 60 F-254 silica gel plates with visualization by UV-light using chloroform and methanol as solvent system. Melting points were determined on a SMP II apparatus (Gehrden, Germany). The IR spectra were recorded on a Shimadzu FTIR 8400S spectrometer (Kyoto, Japan). ¹H NMR spectra were recorded on Bruker Avance-DPX-400 spectrometer (Brillierica, MA, USA) in d₆-DMSO. Chemical shifts were recorded in parts per million downfield from TMS. The splitting patterns of ¹H-NMR were designed as follows: s: singlet, d: doublet, t: triplet, q: quartet, m: multiplet. Mass spectra were recorded on LC-MS-Waters 2695 Alliance Micromass ZQ (Milford, USA). Elemental analysis was performed on Leco CHNS-932 analyzer (Michigan, USA).

All chemicals and solvents were procured from Merck and Aldrich (Darmstad and Steinheim, Germany).

CHEMISTRY

The synthesis of some isatin hydrazide-hydrazones of 4-[[substitutedphenyl] carbamoyl]amino}phenyl)acetic acid is illustrated and outlined in Scheme 1.

General procedure for synthesis of urea derivatives of 4-aminophenylacetic acid [1a-h]

0.500 g (3.3 mmol) 4-(aminophenyl)acetic acid was solved in acetone at 100°C. Then, a solution of corresponding isocyanate (3.3 mmol) in 5 mL acetone was added as three parts per 30 minutes. After 6-8 hours, reaction was finalized by TLC control. Solid material was filtered and recrystallized with a suitable solvent (**1a-h**). Spectroscopic characterisation of **1a-h** have been previously described [8].

General procedure for synthesis of hydrazide-hydrazones of 4-[[substitutedphenyl] carbamoyl]amino}phenyl)acetic acid

Urea derivatives of 4-aminophenylacetic acid [**1a-h**] were esterified with ethanol using sulfuric acid as catalyst and the resulting ester [**2a-h**] was refluxed with hydrazine hydrate in ethanol to give phenylacetyl hydrazines [**3a-h**]. Equimolar amounts of phenylacetyl hydrazines and isatin were refluxed in absolute ethanol for 4-6 hours. The reaction mixture was concentrated and left to cool. The solid product obtained was filtered and recrystallized with ethanol to give as yellow crystals [**4a-h**].

1-(4-Methoxyphenyl)-3-(4-{2-oxo-2-[2-(2-oxo-1,2-dihydro-3H-indol-3-ylidene) hydrazinyl]ethyl}phenyl)urea (**4a**)

IR ν, cm⁻¹: 3318 (NH), 1692, 1633 (C=O), 1430 (C=N). ¹H-NMR (400 MHz, DMSO-d₆, TMS): δ 3.48 (2H, s, -CH₂), 3.70 (3H, s, -OCH₃), 6.81-7.50 (12H, m, Ar-H) 7.86 (s, 1H, N=CH), 8.43 (1H, s, urea NH), 8.53, (1H, s, urea NH), 11.15 (bs, 1H, hydrazone NH), 13.98 (1H, s, indole NH). ¹³C-NMR (100 MHz, DMSO-d₆, TMS) δ (ppm): 165.0 (indole C=O), 161.1 (hydrazide-hydrazone C=O) 154.9 (urea C=O), 114.4, 125.0, 126.4, 129.4, 131.0, 133.2 (aromatic C), 55.60 (CH₃), 36.60 (CH₂). LC/MS (APCI, DMSO-d₆): [M+H] = m/z 444 (%100). Anal. Calcd. for C₂₄H₂₁N₅O₄: C, 65.00; H, 4.77; N, 15.79. Found: C, 65.04; H, 5.22; N, 15.10.

1-(4-Nitrophenyl)-3-(4-{2-oxo-2-[2-(2-oxo-1,2-dihydro-3H-ylidene)hydrazinyl] ethyl}phenyl)urea (**4b**)

IR ν , cm^{-1} : 3100, (NH); 1688, 1630 (C=O), 1410 (C=N). $^1\text{H-NMR}$ (400 MHz, DMSO-d_6 , TMS): δ 3.25 (2H, s, $-\text{CH}_2$), 7.11-8.00 (12H, m, Ar-H) 8.10 (s, 1H, N=CH), 8.70 (1H, s, urea NH), 9.33 (1H, s, urea NH), 11.15 (s, 1H, hydrazone NH), 13.90 (1H, s, indole NH). $^{13}\text{C-NMR}$ (100 MHz, DMSO-d_6 , TMS) δ (ppm): 166.1 (indole C=O), 161.0 (hydrazide-hydrazone C=O), 156.1 (urea C=O), 118.2, 123.89, 126.62, 126.75, 126.79, 127.93, 128.99, 131.14, 131.46 (aromatic C), 36.61 (CH_2). LC/MS (APCI, DMSO-d_6): $[\text{M}+\text{H}] = m/z$ 459 (%100). Anal. Calcd. for $\text{C}_{23}\text{H}_{18}\text{N}_6\text{O}_5$: C, 60.26; H, 3.96; N, 18.33. Found: C, 60.04; H, 3.69; N, 18.10.

1-(4-Trifluoromethylphenyl)-3-(4-{2-oxo-2-[2-(2-oxo-1,2-dihydro-3H-ylidene) hydrazinyl]ethyl}phenyl)urea (**4c**)

IR ν , cm^{-1} : 3106, (NH); 1670, 1635 (C=O), 1425 (C=N). $^1\text{H-NMR}$ (400 MHz, DMSO-d_6 , TMS): δ 3.26 (2H, s, $-\text{CH}_2$), 6.70-7.80 (12H, m, Ar-H), 7.90 (s, 1H, N=CH), 8.25 (1H, s, urea NH), 8.90, (1H, s, urea NH), 12.13 (s, 1H, hydrazone NH), 13.95 (1H, s, indole NH). $^{13}\text{C-NMR}$ (100 MHz, DMSO-d_6 , TMS) δ (ppm): 169.4 (indole C=O), 164.0 (hydrazide-hydrazone C=O), 159.68 (CF_3), 153.0 (urea C=O), 115.00, 123.89 126.62, 126.75, 126.79, 127.93, 128.99, 131.14, 131.46, 136.20, 136.29, (aromatic C), 36.60 (CH_2). LC/MS (APCI, DMSO-d_6): $[\text{M}+\text{H}] = m/z$ 482 (%100.0). Anal. Calcd. for $\text{C}_{24}\text{H}_{18}\text{F}_3\text{N}_5\text{O}_3$: C, 59.88; H, 3.77; N, 14.55. Found: C, 60.02; H, 3.60; N, 14.70.

1-(4-Methylphenyl)-3-(4-{2-oxo-2-[2-(2-oxo-1,2-dihydro-3H-ylidene)hydrazinyl]ethyl} phenyl)urea (**4d**)

IR ν , cm^{-1} : 3106, (NH); 1670, 1635 (C=O), 1425 (C=N). $^1\text{H-NMR}$ (400 MHz, DMSO-d_6 , TMS): δ 2.23 (3H, s, $-\text{CH}_3$), 3.40 (2H, s, $-\text{CH}_2$), 6.70-7.85 (12H, m, Ar-H), 8.05 (s, 1H, N=CH), 8.25 (1H, s, urea NH), 8.90, (1H, s, urea NH), 12.13 (s, 1H, hydrazone NH), 13.95 (1H, s, indole NH). $^{13}\text{C-NMR}$ (100 MHz, DMSO-d_6 , TMS) δ (ppm): 163.0 (indole C=O), 158.6 (hydrazide-hydrazone C=O), 153.0 (urea C=O), 118.1, 125.0, 126.4, 126.70, 126.81, 127.93, 129.4, 131.0, 133.2 (aromatic C), 36.60 (CH_2), 22.40 (CH_3). LC/MS (APCI, DMSO-d_6): $[\text{M}+\text{H}] = m/z$ 428 (%100.0). Anal. Calcd. for $\text{C}_{24}\text{H}_{21}\text{N}_5\text{O}_3$: C, 67.44; H, 4.95; N, 16.38. Found: C, 68.02; H, 4.75; N, 16.40.

1-(4-Chlorophenyl)-3-(4-{2-oxo-2-[2-(2-oxo-1,2-dihydro-3H-indol-3-ylidene)hydrazinyl]ethyl}phenyl)urea (**4e**)

IR ν , cm^{-1} : 3210 (NH); 1684, 1630 (C=O), 1440 (C=N).

$^1\text{H-NMR}$ (400 MHz, DMSO-d_6 , TMS): δ 3.48 (2H, s, $-\text{CH}_2$), 6.70-7.80 (12H, m, Ar-H), 7.95 (s, 1H, N=CH), 8.20 (1H, s, urea NH), 8.95 (1H, s, urea NH), 12.00 (s, 1H, hydrazone NH), 13.90 (1H, s, indole NH). $^{13}\text{C-NMR}$ (100 MHz, DMSO-d_6 , TMS) δ (ppm): 174.0 (indole C=O), 166.2 (hydrazide-hydrazone C=O) 159.2 (C=O urea), 120.0, 122.3, 126.4, 126.9, 127.1, 129.5, 130.2, 132.0 (aromatic C), 36.00 (CH_2). LC/MS (APCI, DMSO-d_6): $[\text{M}+\text{H}] = m/z$ 448 (%100.0). Anal. Calcd. for $\text{C}_{23}\text{H}_{18}\text{ClN}_5\text{O}_3$: C, 61.68; H, 4.05; N, 15.64. Found: C, 61.02; H, 3.95; N, 15.70.

1-(2,6-Dichlorophenyl)-3-(4-{2-oxo-2-[2-(2-oxo-1,2-dihydro-3H-ylidene)hydrazinyl] ethyl}phenyl)urea (**4f**)

IR ν , cm^{-1} : 3100 (NH); 1688, 1640 (C=O), 1410 (C=N). $^1\text{H-NMR}$ (400 MHz, DMSO-d_6 , TMS): δ 3.49 (2H, s, $-\text{CH}_2$), 7.09-7.60 (11H, m, Ar-H), 7.85 (s, 1H, N=CH), 8.10 (1H, s, urea NH), 8.90 (1H, s, urea NH), 12.00 (s, 1H, hydrazone NH), 13.50 (1H, s, indole NH). $^{13}\text{C-NMR}$ (100 MHz, DMSO-d_6 , TMS) δ (ppm): 167.20 (indole C=O), 165.00 (hydrazide-hydrazone C=O) 158.60 (urea C=O), 128.76, 129.14, 129.85, 130.58, 130.75, 131.35, 133.30, 134.26, 135.61, 137.14 (Aromatic C), 35.00 (CH_2). LC/MS (APCI, DMSO-d_6): $[\text{M}+\text{H}] = m/z$ 483 (%100.0). Anal. Calcd. for $\text{C}_{23}\text{H}_{17}\text{Cl}_2\text{N}_5\text{O}_3$: C, 57.27; H, 3.55; N, 14.52. Found: C, 58.10; H, 3.65; N, 14.40.

1-(3,5-Dichlorophenyl)-3-(4-{2-oxo-2-[2-(2-oxo-1,2-dihydro-3H-indol-3-ylidene)hydrazinyl]ethyl}phenyl)urea (**4g**)

IR ν , cm^{-1} : 3150 (NH); 1670, 1640 (C=O), 1410 (C=N). $^1\text{H-NMR}$ (400 MHz, DMSO-d_6 , TMS): δ 3.48 (2H, s, $-\text{CH}_2$), 6.99-8.03 (12H, m, Ar-H and N=CH), 8.20 (1H, s, urea NH), 8.80 (1H, s, urea NH), 12.10 (s, 1H, hydrazone NH), 13.60 (1H, s, indole NH). $^{13}\text{C-NMR}$ (100 MHz, DMSO-d_6 , TMS) δ (ppm): 168.80 (indole C=O), 163.00 (hydrazide-hydrazone C=O) 154.00 (urea C=O), 121.00, 127.96, 128.31, 129.15, 129.91, 130.59, 133.40 (aromatic C), 36.00 (CH_2). LC/MS (APCI, DMSO-d_6): $[\text{M}+\text{H}] = m/z$ 483 (%100.0). Anal. Calcd. for $\text{C}_{23}\text{H}_{17}\text{Cl}_2\text{N}_5\text{O}_3$: C, 57.27; H, 3.55; N, 14.52. Found: C, 57.20; H, 3.60; N, 14.50.

1-(4-{2-Oxo-2-[2-(2-oxo-1,2-dihydro-3H-indol-3-ylidene) hydrazinyl]ethyl}phenyl)-3-(2,4,6-trichlorophenyl)urea (**4h**)

IR ν , cm^{-1} : 3150 (NH); 1670, 1640 (C=O), 1410 (C=N). $^1\text{H-NMR}$ (400 MHz, DMSO-d_6 , TMS): δ 3.48 (2H, s, $-\text{CH}_2$), 6.99-7.83 (10H, m, Ar-H), 8.10 (s, 1H, N=CH), 8.22 (1H, s, urea NH), 8.94 (1H, s, urea NH), 12.00 (s, 1H, hydrazone NH), 13.40 (1H, s, indole NH). $^{13}\text{C-NMR}$ (100

MHz, DMSO- d_6 , TMS) δ (ppm): 165.90 (indole C=O), 164.00 (hydrazide-hydrazone C=O) 152.00 (urea C=O), 121.10, 128.28, 129.73, 130.17, 131.66, 133.0, 133.91, 134.01, 136.21, 137.62 (aromatic C), 36.00 (CH_2). LC/MS (APCI, DMSO- d_6): $[M+H]^+ = m/z$ 517 (%100). Anal. Calcd. for $C_{23}H_{16}Cl_3N_5O_3$: C, 53.46; H, 3.12; N, 13.55. Found: C, 54.10; H, 3.20; N, 13.50.

Larvicidal activity

Larvae of *Ae. aegypti* (Orlando strain) used in these bioassays were hatched from the eggs obtained from a laboratory colony maintained at the Mosquito and Fly Research Unit at the Center for Medical, Agricultural and Veterinary Entomology, USDA-ARS, Gainesville, Florida. The eggs were hatched and the larvae were maintained at a temperature of $27 \pm 2^\circ C$ and $60 \pm 10\%$ RH in a photoperiod regimen of 12:12 h (L: D). Bioassays were conducted using the system described by Pridgeon et al. [21] to determine the larvicidal activity of these compounds against *Ae. aegypti*. Five 1-d-old larvae were transferred to individual wells of a 24-well tissue culture plates in a 30-40 μL droplet of water. Fifty μL of larval diet of 2% slurry of 3:2 beef liver powder (Now Foods, Bloomington, Illinois) and Brewer's yeast (Lewis Laboratories Ltd., Westport, CT) and 1 mL of deionized water were added to each well by using a Finnpiptette stepper (Thermo Fisher, Vantaa, Finland). Compounds were dissolved and diluted in DMSO. Eleven microliters of the test chemical was added to the labeled wells, while 11 μL of DMSO was added to the control treatments. After treatment application, the plates were swirled in clock-wise and counter clockwise motions and front and back and side to side five times to ensure even mixing of the tested compounds. Permethrin (46.1% *cis* – 53.2% *trans*, Chemical Service, West Chester, PA) at 0.025 ppm was used as positive control. Larval mortality was recorded 24 h post treatment. For dose response study, a series of 5 dosages of 4-[(substitutedphenyl) carbamoyl]amino}phenyl)acetic acid derivatives were used to get a range of mortality between 0 and 100%. Treatments were replicated 10 times. LC_{50} values for larvicidal data were calculated by using SAS, Proc Probit [22] (Version 9.2, 2007) Control mortality was corrected by using Abbott's formula [23].

Adulticidal activity

Pupae from the *Aedes aegypti* "Orlando" strain were allowed to emerge into a screen cage with access to 10% sucrose soaked cotton balls. Three to five day old adults were aspirated and cold anesthetized by holding in a 4C

Table 1. Some properties of compounds **4a-h**

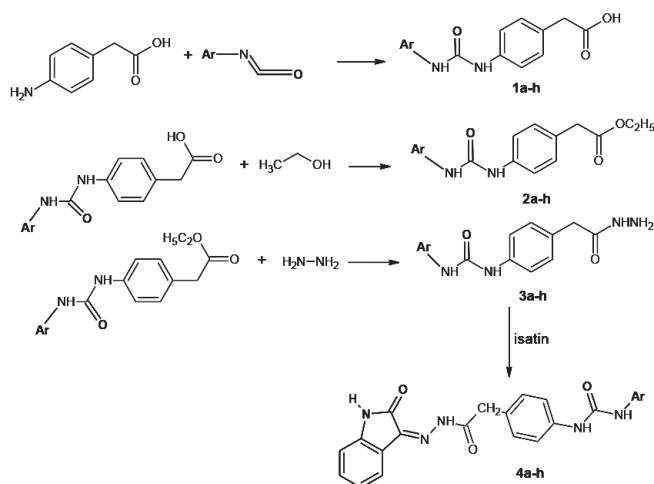
Comp.	Ar	Molecular Formula	Melting point ($^\circ C$)	Yield (%)	Molecular weight
4a	4-OCH ₃ -C ₆ H ₄	C ₂₄ H ₂₁ N ₅ O ₄	258-260	78	443
4b	4-NO ₂ -C ₆ H ₄	C ₂₃ H ₁₈ N ₆ O ₅	250-252	65	458
4c	4-CF ₃ -C ₆ H ₄	C ₂₄ H ₁₈ FN ₅ O ₃	212-214	64	481
4d	4-CH ₃ -C ₆ H ₄	C ₂₄ H ₂₁ N ₅ O ₃	228-230	71	427
4e	4-Cl-C ₆ H ₄	C ₂₃ H ₁₈ ClN ₅ O ₃	200-202	64	448
4f	2,6-Cl ₂ -C ₆ H ₃	C ₂₃ H ₁₇ Cl ₂ N ₅ O ₃	249-251	75	482
4g	3,5-Cl ₂ -C ₆ H ₃	C ₂₃ H ₁₇ Cl ₂ N ₅ O ₃	236-238	78	482
4h	2,4,6-Cl ₃ -C ₆ H ₂	C ₂₃ H ₁₆ Cl ₃ N ₅ O ₃	240-242	73	516

refrigerator. Cohorts of ten female mosquitoes were sorted into 3.5 oz (TK35, Solo) cups and kept on ice until topical application of 0.5ul of test solution. The adult topical assay follows the method described previously [24]. Mortality was recorded after 24 hours for all dilutions as well as the controls. Negative controls consisted of untreated mosquitoes and acetone only treated cohorts. The positive control in this assay was permethrin, with four doses of 1000, 100, 10, and 1 ppm. For the hydrazide-hydrazone derivatives, **4a-h**, a ten-fold dilution series of 10000, 1000, 100, and 10 ppm was made in acetone from a 100000 ppm stock of each compound. Replicates of the same assay were performed on three successive days. Mortality was converted to percentage for each replicate and the three percentage values were used to determine the mean and standard error.

RESULTS AND DISCUSSION

A series of new isatin hydrazide-hydrazone derivatives were prepared according to Scheme 1. The structures of the target compounds **4a-h** were elucidated using UV, IR, 1H -NMR, ^{13}C -NMR and mass spectroscopic methods besides elemental analysis. All compounds were isolated in satisfactory yields (64-78%) and purified by recrystallization from ethanol. The purity of the compounds was checked by TLC and elemental analysis. The chemical structures of all compounds were characterized by various spectroscopic methods and elemental analysis. Both analytical and spectral data of all the synthesized compounds were in full agreement with the proposed structures.

The IR spectra of **4a-h** showed hydrazone N-H bands in 3318-3100 cm^{-1} , C=O bands in 1692-1630 cm^{-1} and C=N bands in 1440-1410 cm^{-1} . 1HNMR spectra of **4a-h** showed



Scheme 1. Synthetic route of the targeted compounds

two signals in the 8.10-7.85 ppm and 12.13-11.15 ppm which are attributed to the N=CH and hydrazone NH protons, respectively. In addition, the N-H peak of indole and urea were observed at 13.98-13.40 ppm and 9.33-7.90 ppm respectively. The signal of aromatic protons was also found in the expected field.

The hydrazide-hydrazone derivatives **4a-h** were evaluated for the first time against yellow fever mosquito, *Ae. aegypti* for their larvicidal activity. Compounds **4a-h** were screened in larval bioassays at concentrations of 100, 50 and 25 ppm in a dose dependent manner and data for the mortality was recorded. Only compound **4b**, having nitrophenyl ring, showed the activity whereas all the other compounds were inactive at the highest dose of 100 ppm. Dose-response data of compound **4b** showed LC_{50} and LC_{90} values of 30.5 (15.4-22.7) and 95.9 (73.8-139.4) ppm, respectively.

To further evaluate the efficacy of **4a-h**, we performed adult topical bioassays using 3-5 day old *Aedes aegypti* females. The permethrin positive control was tested at 1000, 100, 10, and 1 ppm which produced greater than 95% mortality at the three highest doses and 33.3% (Table 2) mortality at 1 ppm. Based on the much larger body mass of the adult as compared to first instar larva, we started with higher doses of 10000, 1000, 100, and 10 ppm for **4a-h**. All compounds, with the exception of **4f** and **4g**, showed greater than 50% mortality at the highest dose of 10000 ppm. At this same dose, treatment with **4a** and **4b** resulted in 80% mortality while **4d** produced complete mortality. However the mortality of **4a-h** tapered quickly and at 1000 ppm was less than 25% for all compounds. Mortality in the untreated and acetone treated negative controls was 6.67% and 10% respectively.

Table 2. 24 hour mortality of compounds **4a-h** on 3-5 day old female *Aedes aegypti*

Comp.	10000 ppm	1000 ppm	100 ppm	10 ppm	1 ppm
4a	80.0±10.0	20.0±15.3	13.3±6.7	20±10.0	Not tested
4b	80.0±20.0	16.7±3.3	3.3±3.3	10.0±5.8	Not tested
4c	70.0±10.0	20.0±5.8	10.0±10.0	16.7±12.0	Not tested
4d	100.0	3.3±3.3	25.0±5.0	6.7±3.3	Not tested
4e	66.7±14.5	10.0±5.8	6.7±6.7	13.3±3.3	Not tested
4f	26.7±14.5	23.2±12.0	10.0±5.8	16.67±12.0	Not tested
4g	40.0±15.3	20.0±15.3	3.3±3.3	6.7±3.3	Not tested
4h	76.7±6.7	20.0±15.3	46.7±23.3	6.7±3.3	Not tested
Permethrin*	Not tested	100	96.7±3.3	96.7±3.3	33.3±17.6

*The permethrin positive control was tested at 1000, 100, 10, and 1 ppm which produced greater than 95% mortality at the three highest doses and 33.3% mortality at 1 ppm.

CONCLUSION

A series of novel isatin hydrazide-hydrazone derivatives were synthesized and evaluated for their larvicidal activity against *Ae. aegypti*. Among the tested compounds, compound **4b**, showed larvicidal activity whereas all the other compounds were inactive at the highest screening dose of 100 ppm. Adult topical bioassays showed greater than 80% mortality at 10000 ppm for compounds **4a**, **4b**, and **4d**, however, these same compounds were relatively inactive at lower doses. These data indicate that compound **4b** may be designed for the new hydrazone derivatives to increase the bioefficacy of these compounds against *Ae. aegypti* larvae.

CONFLICT OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

Acknowledgements

This study was supported by Marmara University Scientific Research Projects Commission [Grant SAG-D-150513-0158] and Deployed War-Fighter Protection Research Program Grant which was funded by the U.S. Department of Defense through the Armed Forces Pest Management Board. We thank the Mosquito and Fly Research Unit, Center for Medical, Agricultural and Veterinary Entomology, USDA-ARS, Gainesville, for supplying *Ae. aegypti* eggs.

Bazı Hidrazit-Hidrazon Türevlerinin Sentezi ve Larvasidal Etkileri

ÖZET

Bir seri hidrazit-hidrazon türevi bileşik sentezlenmiş ve bunların larvisidal ve yüzeyel etkisi *Aedes aegypti* türüne karşı ölçülmüştür. Sentezlenen tüm bileşiklerin yapıları elementel analiz, UV, IR, ¹H-NMR, ¹³C-NMR ve kütle spektroskopisi kullanılarak doğrulanmıştır. **4a-h** bileşiklerinin biyolojik analizleri 100, 50 and 25 ppm

konsantrasyonlarında taranmış ve mortalite oranları kaydedilmiştir. Test edilen bileşikler arasında 1-(4-nitrofenil)-3-(4-{2-okso-2-[2-(2-okso-1,2-dihidro-3H-iliden)hidrazinil] etil} fenil)üre (**4b**) *Aedes aegypti* türüne karşı kaydedeğer larvisidal aktivite göstermiştir. **4b** bileşiğinin doz yanıt verileri, LC₅₀ ve LC₉₀ değerlerini sırasıyla 30.5 (15.4 – 22.7) ve 95.9 (73.8 – 139.4) ppm olarak göstermiştir. Taranan **4a-h** bileşiklerinin dört dozla yapılan biyolojik analizinde aktivite aralığının 10000 ve 1000 ppm arasında olduğu belirlenmiştir.

Anahtar kelimeler: Hidrazit-hidrazon, sivrisinek kontrolü, *Aedes aegypti*, larvasidal etki, yüzeyel etkinlik

REFERENCES

- Katritzky AR, Wang Z, Slavov S, Tsikolia M, Dobchev D, Akhmedov NG, Hall CD, Bernier UR, Clark GG, Linthicum KJ. Synthesis and bioassay of improved mosquito repellents predicted from chemical structure. P.N.A.S. 2008; 105:7359-64.
- Hoel D, Pridgeon JW, Bernier UR, Chauhan K, Meepagala K, Cantrell C. Departments of Defense and Agriculture team up to develop new insecticides for mosquito control. Wingbeats 2010; 21: 19-34.
- Carroll JF, Tabanca N, Kramer M, Elejalde NM, Wedge DE, Bernier UR, Co M, Becnel J, Demirci B, Başer KHC, Zhang S. Essential oils of *Cupressus funebris*, *Juniperus communis*, and *J. Chinensis* (Cupressaceae) as repellents against ticks (Acari: Ixodidae) and mosquitoes (Diptera: Culicidae) and as toxicants against mosquitoes. J. Vector. Ecol. 2011; 36: 258-268.
- Tabanca N, Bernier UR, Ali A, Wang M, Demirci B, Blythe EK, Khan SI, Başer KHC, Khan IA. Bioassay-guided investigation of two monarda essential oils as repellents of yellow fever mosquito aedes aegypti. J Agric Food Chem 2013; 61: 38573-80.
- Tabanca N, Bernier UR, Tsikolia M, Becnel JJ, Sampson B, Werle C, Demirci B, Başer KHC, Blythe EK, Pounders C, Wedge DE. *Eupatorium capillifolium* Essential Oil: Chemical composition, antifungal activity, and insecticidal activity. Nat Prod Commun 2010; 5: 1409-15.
- Tabanca N, Abbas A, Bernier UR, Khan IA, Kocyigit-Kaymakcioglu B, Oruc-Emre EE, Unsalan S, Rollas S. Biting deterrence and insecticidal activity of hydrazide-hydrazone and their corresponding 3-acetyl-2,5-disubstituted-2,3-dihydro-1,3,4-oxadiazoles against *Aedes aegypti*. Pest Manag Sci 2013; 69: 703-8.
- Tabanca N, Wedge DE, Ali S, Khan IA, Kaplancikli ZA, Altintop MD. Antifungal, mosquito deterrent, and larvicidal activity of N-(benzylidene)-3-cyclohexylpropionic acid hydrazide derivatives. Med Chem Res 2013; 22: 2602-9.
- Kocyigit-Kaymakcioglu B, Celen AO, Tabanca N, Ali A, Khan SI, Khan IA, Wedge DE. Synthesis and biological activity of substituted urea and thiourea derivatives containing 1,2,4-triazole moieties. Molecules 2013; 8: 3562-76.
- Narasimhan B, Kumar P, Sharma D. Biological activities of hydrazide derivatives in the new millennium. Acta Pharma Sci 2010; 52: 169-80.
- Olayinka A, Obafemi C, Nwinyi O, Akinpelu D. Microwave assisted synthesis and antimicrobial activity of 2-quinoxalinone-3-hydrazone derivatives. Bioorg Med Chem 2010; 18: 214-21.
- Abdel-Aal MT, El-Sayed WA, El-Ashry EH. Synthesis and antiviral evaluation of some sugar arylglycinoylhydrazones and their oxadiazoline derivatives. Arch Pharm Chem Life Sci 2006; 339: 656-63.
- Koçyiğit-Kaymakcioglu B, Oruç E, Unsalan S, Rollas S. Synthesis and antituberculosis activity of hydrazide-hydrazone. Med Chem Res 2009; 118: 277-86.
- Rostom SAF, Shalaby MA, El-Demellawy MA. Polysubstituted pyrazoles, part 5. Synthesis of new 1-(4-chlorophenyl)-4-hydroxy-1H-pyrazole-3-carboxylic acid hydrazide analogs and some derived ring systems. A novel class of potential antitumor and anti-HCV agents. Eur J Med Chem 2003; 38: 959-74.
- Koçyiğit-Kaymakcioglu B, Oruc E, Unsalan S, Kandemirli F, Shvets N, Rollas S, Dimoglo A. Synthesis and characterization of novel hydrazide-hydrazone and the study of their structure-antituberculosis activity. Eur J Med Chem 2006; 41: 1253-61.
- Koçyiğit-Kaymakcioglu B, Oruç-Emre EE, Unsalan S, Tabanca N, Wedge DE, Khan SI, Demirci F, Rollas S. Synthesis and biological activity of hydrazide-hydrazone and their corresponding 3-acetyl-2,5-disubstituted-2,3-dihydro-1,3,4-oxadiazoles. Med Chem Res 2012; 21: 3499-508.
- Boger M, Dur D, Gsell L, Hall RG, Karrer F, Kristiansen O, Maiefisch P, Pascual A, Rindlisbacher A. Synthesis and structure activity relationships of benzophenone hydrazone derivatives with insecticidal activity. Pest Manag Sci 2001; 57: 191-202.
- Liu M, Wang Y, Wangyang WZ, Liu F, Cui YL, Duan YS, Wang M, Liu SZ, Rui CH. Design, synthesis and insecticidal activities of phthalamides containing a hydrazone substructure. J Agric Food Chem 2010; 58: 6859-63.
- Wu J, Song BA, Hu DY, Yue M, Yang S. Design synthesis and insecticidal activities of novel pyrazole amides containing hydrazone substructures. Pest Manag Sci 2012; 68: 801-10.
- Aggarwal G, Kumar R, Srivastva C, Dureja P, Khurana JM. Synthesis of nalidixic acid based hydrazones as novel pesticides. J Agric Food Chem 2010; 58: 3056-61.
- Yanga C, Shao Y, Zhi X, Huana Q, Yua X, Yao X, Xu H. Semisynthesis and quantitative structure-activity relationship (QSAR) study of some cholesterol-based hydrazone derivatives as insecticidal agents. Bioorg Med Chem 2013; 23: 4806-12.
- Pridgeon JW, Becnel JJ, Clark GG, Linthicum KJ. A high throughput screening method to identify potential pesticides for mosquito control. J Med Entomol 2009; 46: 335-41.
- SAS Institute. 2007. SAS Online Doc., version 9.2. SAS Institute, Cary, NC.
- Abbott WS. A method of computing the effectiveness of an insecticide. J Econ Entomol 1925; 18: 265-7.
- Pridgeon JW, Becnel JJ, Clark GG, Linthicum KJ. Permethrin induces overexpression of multiple genes in *Aedes aegypti*. J Med Entomol 2009; 46: 580-7.